U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY—BULLETIN NO. 60.

B. T. GALLOWAY, *Chief of Bureau.*

A SOFT ROT OF THE CALLA LILY.

BY

C. O. TOWNSEND, PATHOLOGIST.

VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS.

ISSUED JUNE 30, 1904.

WASHINGTON:
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BULLETINS OF THE BUREAU OF PLANT INDUSTRY.

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THIRD CROP OF HEALTHY CALLAS GROWN IN THE SAME SOIL ACCORDING TO METHODS ADVOCATED IN THIS BULLETIN.

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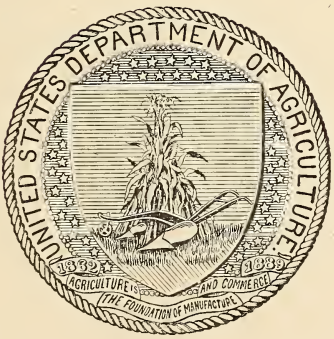

WASHINGTON:
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1904.

BUREAU OF PLANT INDUSTRY.

B. T. Galloway, *Chief.*
J. E. Rockwell, *Editor.*

VEGETABLE PATHOLOGICAL AND PHYSIOLOGICAL INVESTIGATIONS.

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[a] Detailed to the Bureau of Forestry.
[b] Detailed to Botanical Investigations and Experiments.

LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Plant Industry,
Office of the Chief,
Washington, D. C., March 31, 1904.

Sir: I have the honor to transmit herewith the manuscript of a technical paper submitted by the Pathologist and Physiologist on "A Soft Rot of the Calla Lily," by Dr. C. O. Townsend, Pathologist, Vegetable Pathological and Physiological Investigations, and recommend its publication as Bulletin No. 60 of the series of this Bureau. The accompanying nine plates and seven figures are necessary to a clear understanding of the subject-matter of the text.

Respectfully,

B. T. Galloway,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

PREFACE.

Growers of the calla lily have suffered serious losses for several years from a soft rot which frequently destroys the plants just before or during the flowering period. A bacillus has been separated from the decayed portion of the calla in pure cultures and by repeated inoculations has been shown to be the cause of this destructive disease.

In addition to the principal morphological and physiological characters of the organism which are described in this bulletin, several preventive measures are suggested which have been found to be satisfactory in holding the disease under control. As the bacillus producing this disease is also capable of attacking many of our food plants, growers of vegetables should guard against any possible contamination of the soil with it.

A. F. Woods,
Pathologist and Physiologist.

Office of Vegetable Pathological
and Physiological Investigations,
Washington, D. C., March 30, 1904.

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A SOFT ROT OF THE CALLA LILY.

INTRODUCTION.

Under favorable conditions the calla lily has heretofore been one of the most satisfactory plants produced either in the open or under glass. In most parts of the United States the calla will grow out of doors and will live and thrive from year to year even in the northern latitudes, especially if the corms[a] are protected during the winter season. As a marketable product, however, it is more profitable if grown under glass, where under proper conditions the plants may be forced and the flowers consequently produced in great abundance at the time when they will be in greatest demand. It is under these conditions of forced growth that the plants seem to be most susceptible to disease.

The profits which arise from calla growing are derived either from the sale of the corms or of the flowers, or from both. A bed of a thousand corms, for example, will under normal conditions produce 5,000 flowers, which ordinarily will sell for about $1,000. The corms are grown either in solid beds or in pots. As a rule the best results both as regards the size and the number of flowers produced are obtained from the solid bed. The flowers are always delicate and can not be satisfactorily shipped long distances, while the corms, on the other hand, may be transported for thousands of miles without injury.

There are several diseases to which the calla is susceptible, but the most serious one with which the growers have had to contend is the soft rot that forms the subject of this bulletin. This disease has recently made its appearance in the various parts of the United States where callas are cultivated and has caused enormous losses to the growers, rendering the production of this hitherto profitable plant very uncertain.

The soft rot of the calla was brought to the attention of the writer in the autumn of 1899, and it has been under his observation and study since that time. While there are some points that need further

[a] The true botanical name *corm* is used in this bulletin instead of the common but incorrect term *bulb*.

investigation, it has been deemed best to place the following results before the public, with the hope that the suggestions herein contained may be of value to the industry.

CAUSE OF THE CALLA ROT.

Upon examining microscopically the decayed portions of the calla corms myriads of bacteria were found to be present. In order to obtain cultures of the organism in the best possible condition a partly decayed corm was thoroughly washed with tap water, then with corrosive sublimate (1 part in 1,000), and afterwards with distilled water. A small opening was then made with a sterile knife through the sound part of the corm into the inner marginal part of the decayed spot. A little of the soft tissue just at the border between the decayed and healthy portions of the corm was obtained on a sterile needle and placed in sterile beef broth. Agar plates were then made from this culture, and but one kind of colony was obtained, indicating that the organism was present in the recently decayed portion of the corm in a pure culture. A few days after the colonies had formed, subcultures were made in beef broth and minute portions of these were introduced into various parts of healthy callas. The inoculations were made by placing a drop of the beef-broth culture on the part of the plant to be inoculated, and with a sterile needle punctures were made through these drops into the tissues of the plants. For control, punctures were made in similar parts of healthy plants without adding the broth culture. In a few days the inoculated spots had turned brown and decay had begun, while the controls in all cases remained healthy. Plate cultures were again made from the inoculated spots after decay had begun, and apparently the same organism in pure culture was obtained. This process was repeated many times—i. e., until there was no doubt that this organism was the cause of the soft rot of the calla.

Upon looking up the literature regarding calla diseases it was found that Halsted had discovered a soft rot of the calla corm in 1893.[a] Although Halsted's description is very brief, he undoubtedly refers to the same disease as that which forms the subject of this bulletin. He ascribes the cause of the affection to a bacterium which is found in great abundance in the diseased portions of the corm. A disease of similar nature is also mentioned by Selby.[b] This is referred to as a root rot of the calla, and as no description is given either of the disease or of the organism producing it, it is impossible to determine whether this is the disease now under consideration. The soft rot of the calla and the organism producing it have been observed by Dr. Erwin F. Smith, the pathologist in charge of the laboratory of plant pathology of the United States Department of Agriculture, and by Mr.

[a] Diseases of Calla. New Jersey Experiment Station Report for 1893, p. 399.

[b] Selby. Calla. In Condensed Handbook of Diseases of Plants in Ohio, 1900, p. 21.

Newton B. Pierce, the pathologist in charge of the Pacific coast laboratory of the Department, and probably by others, but so far as can be determined it has not hitherto received careful investigation.

GENERAL APPEARANCE OF THE DISEASE.

Several greenhouses where the disease was reported to be present were visited by the writer, who found the callas rotting off usually at or just below the surface of the ground, the disease sometimes extending down into the corm, sometimes upward into the leaves, and frequently in both directions. Occasionally the disease seemed to start in the edge of the leafstalk (fig. 1), in the flower stalk, or in some underground part of the corm, though as a rule it started at the top of the corm just above but near the surface of the ground. It was also noticed that the disease was worse and spread more rapidly in those houses where the callas were grown in solid beds.

FIG. 1.—A slightly diseased calla plant.

When a diseased corm was cut open it was found that there was a distinct line between the healthy and the diseased portion of the corm (fig. 2). The healthy portion of the corm is firm and nearly white, while the diseased part has a decidedly brown color and is soft and watery. When the disease extends upward into the leaves it is the edge of the petiole that first becomes involved, the affected part becoming slimy without immediately losing its green color. As the disease progresses it extends inward toward the center of the petiole and interferes with the transference of material between the corm and the leaf, the edges of the leaf becoming pale, then brown. Pale spots becoming brown then appear in other parts of the leaf blade, and finally the whole leaf becomes brown and dead. Frequently the disease develops so rapidly that the leaf rots off at the base and falls over before it has time to lose its green color. When the disease has progressed far enough to attack the flower stalk, the flower turns brown and the stalk, without having lost its color and frequently without having decayed upward more than a fraction of an inch, eventually falls over. When the disease works downward through the corm it sooner or later reaches the roots, which become soft and slimy within, while the epider-

mis remains intact, thus presenting the appearance of thin-walled tubes filled with a soft substance. The roots remain attached to the corm and eventually the slimy contents dry up and only the dead skin of the roots remains. When the disease begins its attack below the surface of the ground the lower portion of the corm frequently rots away, causing the plant to fall over without having previously given any indication of disease. An examination of the decayed corm shows that only a small part of the upper portion of the corm, with a few side roots, remains. The

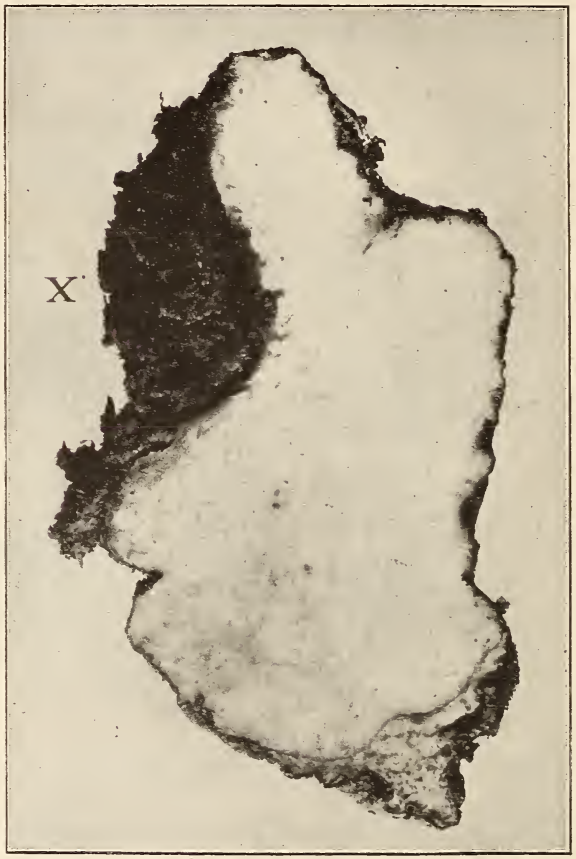

FIG. 2.—A partly decayed calla corm.

latter become less and less numerous as the disease advances, until at last they are unable to support the weight of the leaves and flower stalks.

If the conditions for the development of the disease are unfavorable after the corms are affected, the softened spots will dry down, sinking below the surrounding portion of the corm and becoming darker colored. In these spots the disease will often remain dormant until the conditions for the development of the organism again become favorable. In this way the disease is carried over from season to season, and it may be transported long distances.

EFFECT OF THE ORGANISM ON THE CALLA.

As already stated, the part of the plant usually attacked first is the upper portion of the corm at or just below the surface of the ground. A microscopic examination of the affected part, whether root, corm, leafstalk, or flower stalk, shows that the organisms occupy the intercellular spaces and by some means dissolve the intercellular layer, causing the cells to separate easily, so that when the diseased tissue is placed in a liquid each cell floats out by itself. The cell wall, however, remains intact, but the cell contents are contracted. The rapidity with which the disease advances depends to a large extent upon the external conditions surrounding the plants. Under favorable conditions—a warm

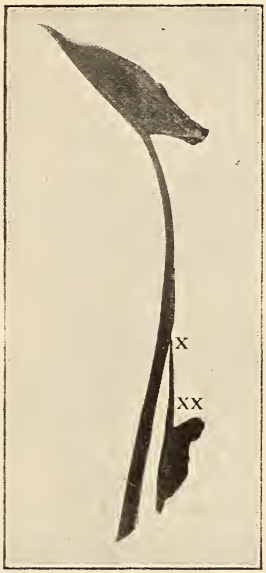

FIG. 3.—Calla leaf twenty-two hours after inoculating with the calla organism. The point of inoculation is shown by X.

FIG. 4.—Calla flower stalk twenty-two hours after inoculating with the calla organism. The point of inoculation is shown by X.

atmosphere and an abundance of moisture—the disease may completely rot the corm in from three to four days, while under less favorable conditions it may be several weeks in destroying the corm, or, indeed, the progress of the disease may be entirely arrested for a period of several months. While the organism usually attacks the corm first, it may also attack either the leafstalk or the flower stalk and cause it to become discolored and decayed. (See figs. 3 and 4.)

MORPHOLOGICAL CHARACTERS OF THE ORGANISM.

The organism which causes the rotting of the calla corm is a very short rod, with rounded ends, as shown in figure 5, and also in Plate II, figure 1. The width of the rods is very nearly uniform.

In a 24-hour-old beef-broth culture they measure about 0.5μ in width. In the same culture the length varies from 2μ to 3μ. The very short ones, as shown by the measurements, are round, or nearly so; these eventually elongate, becoming rods. After the organisms have elongated, cross walls are formed and as a rule they soon break in two, forming separate organisms; but occasionally they remain intact until a long chain is formed, which may finally break up into individual cells (Pl. II, fig. 1). This organism moves with a gliding motion, and upon staining for flagella it is found to possess from two to eight wavy flagella scattered over the surface of the body (Pl. II, fig. 1). The flagella vary in length from 4μ to 18μ, i. e., two to six times the average length of the body. No spores belonging to this organism have been found in any of the artificial cultures or in the diseased plants.

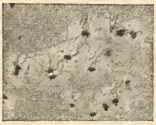

FIG. 5.—*Bacillus aroideæ* with flagella × about 600.

PHYSIOLOGICAL CHARACTERS OF THE ORGANISM.

Certain physiological characters of the organism have been determined by growing it on different media and under various conditions of light, heat, etc., as described in the following pages.

NUTRIENT MEDIA.

In studying the physiology of this organism the following media have been used, viz, beef broth, agar, gelatin, Uschinsky's solution, Dunham's peptone solution, peptone water with rosolic acid, peptone water with methylene blue, simple peptone water,[a] milk, litmus milk, indigo-carmine peptone, and egg albumen. All these culture media were carefully prepared. The beef-broth stock was made from lean beef, the chemicals were "c. p.," and only distilled water was used. In addition to these media the following vegetables and fruits were used, viz, potatoes, onions, turnips, celery, cucumbers, peppers (green fruits), pieplant, beets, radishes, cauliflower, cabbage, eggplant, tomatoes, salsify, carrots, parsnips, apples, pineapples, and bananas.

In nearly all cases both the fruits and vegetables were used raw, but in some instances the vegetables were cooked. Usually the raw fruits and vegetables were sterilized by removing the outer layer with a sterile knife and washing thoroughly with corrosive sublimate (1 part in 1,000) and then with sterile water. They were then cut in thick slices and placed in deep petri dishes and inoculated with one or more loops of a 24-hour beef-broth culture of the organism. When the vegetables were cooked they were cut into cylinders and placed in test tubes with distilled water, then thoroughly sterilized, and when cool inoculated with fresh cultures of the organism.

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[a] Witte's *Peptonum siccum* was the only peptone used.

Beef-broth.—Ten cubic centimeters of standard beef broth inoculated with a 1-mm. loop of a fresh culture fluid of the organism was distinctly clouded in from four to eighteen hours at a temperature of 35° to 18° C. If the temperature was raised or lowered through several degrees above or below the limits indicated or if the inoculation was made from a less active culture, the clouding took place less rapidly. Indeed, the clouding was delayed indefinitely by lowering the temperature to 5° C. or by raising the temperature to 41° C. If the beef broth was kept at room temperature (18° to 24° C.) the organism remained alive for several weeks and a nearly white deposit several millimeters in depth formed in the bottom of the tube.

Agar plate cultures.—On the ordinary nutrient agar poured plates made from a 24-hour-old beef-broth culture colonies were distinctly visible in twenty-four hours at room temperatures of 18° to 20° C., and plates made in the same way and kept at 30° to 35° C. showed colonies distinctly in from fifteen to eighteen hours. The form and size of the colonies on the agar plates depended upon certain conditions—e. g., if the colonies were numerous they were small and round, while if there were but few colonies in each plate they were sometimes round and sometimes radiating. They were usually radiating if the plates were made from fresh cultures and kept at a temperature of from 22° to 35° C. On the other hand, if the plates were made from an old culture or if they were kept at an abnormally high or an abnormally low temperature the colonies were round, even if there were but few in each plate, Agar plate cultures made from Uschinsky's solution or broth cultures that had been kept dormant for several months produced round colonies, but after a few transfers from the dormant state to fresh media the agar plate cultures became characteristically radiating (Pl. II, figs. 2, 3, 4, and 5, and Pl. III, fig. 1).

The foregoing applies to the surface colonies, but in addition to these there were some embedded colonies in practically all poured plates. The embedded colonies were all spindle shaped unless viewed end on, when they appeared to be round, with sharp, distinct outlines. They had a faint yellow tinge, and were much smaller than the surface colonies. If the embedded colonies broke through the surface, they spread out and behaved in the same manner as if they had been originally surface colonies. (See the small colonies on Pl. II, figs. 2 and 5.) Some of the colonies lying at the extreme bottom of the agar—i. e., between the agar and the bottom of the petri dish—spread out, forming a thin layer which eventually gave to the plate a milky appearance when held up to the light. (See Pl. II, fig. 2, and Pl. III, fig. 2.) The surface colonies, whether round or radiating, had a shiny white surface and were only slightly opalescent. If radiating, they usually had a central body, from which the branches radiated (Pl. III, fig. 1). The central body was more dense than the arms or branches and the whole colony was slightly elevated above the sur-

face of the agar. The outlines were sharp and when magnified 125 times the 48-hour-old colonies had a granular appearance.

Agar streak cultures.—In addition to beef broth, peptone, etc., some of the agar tubes contained 5 per cent of grape sugar and others contained 5 per cent of glycerin. These were slanted and inoculated by dipping a sterilized needle in a 24-hour-old beef-broth culture and drawing it lightly over the surface of the slant agar. Streaks became distinctly visible in twenty-four hours at 20° to 25° C. in all the tubes inoculated. The outlines of the streaks were entire at first, but became more or less irregular in from two to four days at 18° to 25° C. Growth was elevated above the surface of the agar and had a shiny appearance, as if wet. It was of a white or grayish-white color and did not discolor the agar nor tend to grow into it. The condensation water became distinctly milky and more or less deposit was formed in it. On the other agars the organism remained alive for several months at room temperatures (20° to 25° C.) if the culture was not allowed to become dry.

Agar stab cultures.—At room temperatures (20° to 25° C.) growth was apparent in from eighteen to twenty-four hours near the top of the stab, and within twenty-eight hours it was distinctly visible throughout the entire length of the stab. The stab increased in size from day to day and in a week was from 1 to 2 mm. in diameter, slightly tapering toward the bottom. The "nail head" gradually increased in size and in from three to five days covered the surface of the agar in the tube. This growth was slightly elevated, grayish-white, with a wet, shining surface and an entire margin. It was thicker in the center, forming a convex layer on the agar. Growth continued for several weeks, with no change in the color of the agar and no change in the stab or line of growth except that it gradually increased in size, retaining its tapering form and its slightly serrate outline with no elongated projections into the agar.

Beef agar, with iron sulphate.—Several slant tubes containing 10 c. c. of nutrient agar plus 1 drop of a saturated solution of ferrous sulphate, and several slant tubes containing 10 c. c. of nutrient agar plus 2 drops of the iron sulphate solution, were inoculated with a fresh culture of the calla organism, while several tubes of each were left for control. In forty-eight hours the organism had spread over the surface of the agar in all inoculated tubes and the inoculated surfaces showed a copious growth for several weeks, but no change was produced in the color of the medium.

Gelatin stab cultures.—These cultures were made with gelatin of different kinds. The first was −10 on Fuller's scale, the second was neutralized with sodium hydroxid, while the third was the same as the second except that another kind of gelatin was used. Growth was apparent within twenty-four hours (at 18° to 22° C.) in all the tubes

inoculated. At the end of twenty-four hours the stabs were distinctly visible throughout their entire length in all the inoculated tubes (Pl. IV, fig. 1, A). In forty-eight hours from the time of inoculation the gelatin in all the tubes began to liquefy (Pl. IV, fig. 1, B). Liquefaction advanced most rapidly in No. 3 and least rapidly in No. 1. In three days No. 3 had entirely liquefied, and in five days No. 1 and No. 2 had also liquefied (Pl. IV, fig. 1, C). After the gelatin had liquefied a cloudy mass floated about in the clear liquid. This finally settled, forming a copious white deposit. The deposit was most abundant in No. 3, but in No. 1 it formed a layer from 2 to 5 mm. deep.

Egg albumen.—Several tubes of solidified egg albumen were inoculated with a fresh culture of the organism, but only a feeble growth appeared and no change had been produced in the color of the albumen at the end of eight weeks.

Milk.—This medium was sterilized by heating for ten minutes at 100° C. in a steam sterilizer on three successive days, the milk having been previously placed in test tubes (10 c. c. in each tube), and the tubes closed with cotton plugs. The milk was inoculated by placing a 1-mm. loop of a 24-hour-old beef-broth culture in each of several of the tubes. The curdling of the milk began to take place in from two to three days in all parts of the inoculated tubes. Two days later the entire 10 c. c. of milk was solidified and a layer of whey about 1 mm. deep rested upon the top of the curd. These experiments were repeated from time to time, with the same results. Whey continued to be separated for several days until from one-third to one-half the space formerly occupied by the milk was occupied by the liquid; but no abnormal coloring was produced in any of the tubes. None of the control tubes curdled in any case.

Litmus milk.—This medium was prepared in the same manner as the milk, except that a few drops of strong litmus solution were added to each tube of milk before sterilizing. Several of the tubes were inoculated with a 1-mm. loop of a 24-hour-old beef-broth culture. Within forty-eight hours the blue began to give way to a reddish color near the surface, which within three days had extended throughout the inoculated tube. At the end of five days from the time of inoculation the red color had decidedly faded throughout, so that the tubes that were litmus blue when inoculated were now only faintly pink, and the milk had curdled throughout. The curdling of the milk and the separation of the whey took place in the same manner as if the litmus had not been present. In nine days even the pink color had disappeared, with the exception of a faint rim near the surface. These discolored litmus tubes were then allowed to stand until the organism had died. The red litmus color, eventually becoming blue, gradually returned, although the milk remained curdled and the whey separated—about one-half whey and one-half curd.

Litmus milk in nitrogen.—It was noticed that the litmus milk tubes, whether they had been inoculated or not, contained a deposit of blue litmus. The calla organism that bleached the litmus in the milk failed to attack this deposit, so that it remained blue. It was suggested that the milk possibly contained an anaerobic bacterium that was not destroyed by sterilizing and that it favored the formation of the blue deposit. The two control tubes of litmus milk were placed in a bottle holding about a quart. The bottom of the bottle was covered with pyrogallic acid (powder) to a depth of about one-half inch. To this 50 c. c. of a 10 per cent solution of caustic potash were added, and the bottle was quickly sealed with Darwin's wax. The mixture was shaken for some time to enable it to take up the oxygen without forming much carbon monoxid. If the deposit were due to an anaerobic bacterium, it should increase farther up in the tubes. At the expiration of twelve months the jar was opened. A lighted match thrust just below the level of the opening in the jar was immediately extinguished, showing that the jar still contained nitrogen and had not allowed oxygen to enter during this time. An examination of the tubes showed that the blue deposit had not changed. This indicated that the deposit was undoubtedly a mechanical one and was not due to the presence of an organism. The inoculated tubes that were left in the ordinary air gradually regained their blue color after the organism died. The return of the color (first red, then blue) was apparent whether the organisms were left to die of their own accord or whether they were destroyed by heating; e. g., if an inoculated litmus tube had entirely faded and was then heated for ten minutes at 100° C., the color returned within twenty-four hours.

Uschinsky's solution.—Several tubes of Uschinsky's solution were inoculated with a 1-mm. loop of a 24-hour-old beef-broth culture. Seventeen hours later at 25° C. all inoculated tubes were slightly clouded. Thirty-six hours after inoculation the tubes were decidedly clouded throughout, with a slight whitish deposit in some of them. The cloudiness was not uniform in all parts of the same tube, but was somewhat stratified. Both the cloudiness and the deposit increased from day to day, until at the end of one week the solution was uniformly clouded, milk-white, with a copious white deposit in the bottom of the tube. Even at the end of three months at normal room temperatures the organism was still alive, as indicated by the fact that the tubes were still clouded and a 1-mm. loop placed in beef-broth caused a distinct clouding in twenty-four hours. At this time the precipitate was 3 mm. deep. Plating and inoculating into callas showed it to be the calla organism. This experiment was repeated several times with identical results both in regard to the clouding of the Uschinsky solution and the longevity of the organism in this medium.

Dunham's solution.—Several tubes of Dunham's solution were inoculated with a 1-mm. loop of a 24-hour-old culture of the calla-rot organism in beef broth. In twenty-four hours at 20° C. a faint cloudiness was perceptible. This increased slightly from day to day for about six days. The temperature during this time ranged from 18° to 25° C. The cloudiness then seemed to remain practically constant for about one week. A deposit was gradually formed, and in one month from the time of inoculation the solution became almost clear, showing that the organism had ceased to live. The deposit formed was about 1 mm. in depth and had a faint brownish tinge.

Dunham's solution, with acid fuchsin.—This solution was inoculated the same as above. At the end of one week the solution in the inoculated tubes was lighter colored than in the control tubes. At the end of one month after inoculation the bleaching seemed to have ceased. The organisms were nearly all dead, as indicated by the fact that the liquid was practically clear. While the solution in the inoculated tubes was somewhat pinkish in color, it was decidedly lighter than the solution in the control tubes. The deposit was the same in color and in quantity as in the Dunham solution given above.

Dunham's solution, with indigo-carmine.—Sterile tubes of this solution were inoculated in the same manner as the Dunham's solution. In two days the inoculated tubes were slightly blue when seen by reflected light. This color deepened from day to day for about one week, after which time it remained practically constant. The inoculated tubes were only slightly clouded at the end of two weeks, and a small quantity of deposit with a faint brownish tinge had formed in the bottom of the inoculated tubes.

Peptone solution, with rosolic acid.—A nutrient solution containing rosolic acid was inoculated with a 1-mm. loop from a 24-hour-old beef-broth culture, and at the end of one week the solution had a milky appearance, due to the presence of a large number of organisms. Ten days later there was no change, except the formation of a small amount of white deposit. At the end of thirty days after inoculation the tubes were still slightly clouded, but no change in color was apparent. The deposit had increased and had assumed a faint brownish tinge.

Dunham's solution, with methylene blue.—Two preparations containing peptone and methylene blue were used. The first consisted of a 1 per cent solution of Witte's peptone, to which was added 0.5 per cent c. p. sodium chlorid and 3 c. c. of a 1 per cent aqueous solution of methylene blue. Sterile tubes of the solution were inoculated with pure 24-hour-old beef-broth cultures of the calla-rot germ. These inoculated tubes were compared with the controls for two months subsequent to inoculation, but no change in color could be detected.

The second preparation was the same as the first, except that it contained 1 per cent of grape sugar. Three days after inoculation there was no apparent change in color, but at the end of five days the inoculated tubes had a greenish tinge. This became more distinct from day to day for several weeks, and at the end of two months the inoculated tubes were entirely green, while the control tubes remained blue. The blue color of the inoculated tubes was not restored upon shaking.

Steamed potato cylinders.—Potato cylinders were sterlized by steaming on three consecutive days in the sterilizer. Some of these were inoculated with a 1-mm. loop of a 24-hour-old culture of the calla-rot organism in beef broth. Twenty-four hours after inoculation the organism had spread over about two-thirds of the slant surface of the inoculated cylinders. The rate of growth was slow as compared with that on other media. The surface of the growth had a shiny appearance and a faint tinge of yellow which corresponded very closely to Ridgway's Cream Color, No. 20, Plate VI, or Saccardo's Cremeus, No. 27, Table II. The inoculated cylinders began to turn gray toward the inoculated ends. Even in twenty-four hours the discoloration extended from one-third to two-thirds of the length of the cylinders. The color deepened from day to day until at the end of two weeks the upper ends of the cylinders were distinctly brown, the color fading into a gray toward the lower ends of the cylinders. All the many inoculated cylinders retained their shape, and the control cylinders remained firm and white throughout the experiment.

In testing the potato cylinders for starch the reaction was immediate in both the inoculated and the control cylinders and the color was nearly the same, but less purple and more blue in the control than in the inoculated tubes. These tests were made at the end of the second week and later. The odor of the inoculated cylinders at the end of two weeks was sour and disagreeable, resembling spoiled paste.

Raw potato.—A fairly smooth potato was selected and thoroughly washed with tap water to remove the surface dirt. It was then washed with distilled water and the surface was sterilized with a solution of corrosive sublimate (1 part in 1,000), after which it was rinsed with sterile water. It was then cut with a sterilized knife into slices about 2 cm. in thickness. Each slice was divided into four parts and placed in a deep sterilized petri dish. Several petri dishes were prepared in this manner. Two of the pieces in each were inoculated with a 24-hour-old beef-broth culture of the calla organism by placing several drops of the beef-broth culture on the surface of the pieces and then stabbing through these drops into the potato with a sterile needle. Two pieces were left for control. In twenty-four hours the inoculated and control pieces showed a slight discoloration owing to the action of the air, but only the inoculated pieces decayed.

At the end of five days the decayed portions closely resembled Ridgway's Broccoli Brown, No. 15, Plate III. It was not quite as dark as Saccardo's Umbrinus, No. 9, Table I. The inoculated pieces had the odor of decaying vegetables and were alkaline to litmus.

Raw eggplant.—A ripe fruit of the eggplant was obtained from the market, the surface was washed and sterilized as described above, and it was then cut with a sterile knife into slices of thickness suitable for placing in petri dishes. In some instances the slices were pared with a sterile knife so as to remove the outside skin, and in other cases the skin was left on. All slices were cut into four pieces, two of which were inoculated with a 24-hour-old culture of the germ in beef broth and two were left for control. Within eighteen hours at from 20° to 24° C. the inoculated pieces were discolored, and in forty-eight hours the discoloration had extended entirely through them. In three days some of the inoculated pieces were somewhat split and shrunken, as shown in Plate IV, figure 2. In color the interior—i. e., the part that was the center of the fruit—was Broccoli Brown, No. 15, Plate III, of Ridgway's tables, a little lighter than Saccardo's Umbrinus No. 9, Table I. The portion toward the margin was nearly Clove Brown, No. 2, Plate III, Ridgway's tables, or a little darker than Saccardo's Castaneus, No. 10, Table I. There was no sharp line between these two shades of brown, but one graded into the other. The inoculated pieces at the end of three days had a decidedly soapy odor and the reaction was alkaline to litmus. The checks remained perfectly sound.

Raw cauliflower.—A large head of cauliflower that had been three weeks in cold storage was obtained from the market. A portion of the main stalk was thoroughly washed with corrosive sublimate, and then with sterile water. With a sterile knife the outside was pared off and the remaining part was then cut into slices that could be conveniently placed in petri dishes. These were then inoculated with the calla-rot germ from a pure culture in beef broth, leaving a number of pieces for control. The culture used in this case was three days old. In twenty hours at 20° to 24° C. the inoculated pieces began to show a faint discoloration, turning slightly brown. This continued until at the end of about two and a half days the whole of each piece inoculated had become discolored. At this time the inoculated pieces were decidedly alkaline in reaction, gave a very strong odor of decaying vegetable matter, and on comparing with Ridgway's plates the color was found to correspond very closely to the Ecru Drab, No. 21, Plate III, or to Saccardo's Avellaneus, No. 7, Table I. The control pieces were still healthy. In several cases the inoculations did not take. Several branches from the head were sterilized and the lower part was inoculated with the same germ. In all these cases the inoculation was successful, with the same characteristic odor, color, and reaction.

Raw radish.—Several red, so-called "white tip," round radishes were obtained from the market. These were washed and the surfaces sterilized in the same manner as the raw potatoes. They were then pared with a sterile knife, cut in half, and placed in petri dishes, four halves in each dish. Immediately after preparing these specimens, two in each dish were inoculated with the calla-rot organism, using a 24-hour-old beef-broth culture, and in eighteen hours at 20° to 25° C. all the inoculated pieces showed slight discoloration. In forty-eight hours the disease had advanced so that the whole of each inoculated piece was discolored. None of the uninoculated pieces showed any signs of disease. Some of the inoculated pieces were inoculated by contact and others by stab. The disease progressed as rapidly in the contact as in the stab cultures. The inoculated pieces only were affected; color, Cinnamon, No. 20, Plate III, Ridgway, a little lighter than Saccardo's Umbrinus, No. 9, Table I. In reaction the discolored pieces were strongly alkaline to litmus, and had the very disagreeable odor of decaying vegetables. All the inoculated pieces were involved (see Pl. V, fig. 1), gradually disintegrated, and settled down upon the bottom of the petri-dishes, as shown in Plate V, figure 2.

Raw cucumbers, sliced.—A green cucumber about 5 inches in length was thoroughly washed with distilled water and the surface sterilized with corrosive sublimate (1 part in 1,000). The outer rind was peeled off with a sterile knife, and the material was then cut into slices from 1½ to 2 cm. in thickness. Each slice was divided into two parts and placed in sterile petri dishes, four pieces in each dish. Two of these pieces in each dish were inoculated with the calla disease germ, using a 24-hour-old beef-broth culture. All the inoculated pieces began to show slight discoloration in eighteen hours at 20° to 25° C., and in forty-eight hours the disease had progressed rapidly, having discolored in some cases the whole of each inoculated piece. The color of the inoculated pieces at this time was light brown or yellowish, closely resembling Ridgway's Buff, No. 13, Plate V, or Saccardo's Ochroleucus, No. 28, Table II. The inoculated pieces had a peppery, pungent odor, and were strongly alkaline to litmus.

Raw cucumbers, whole.—The effect of the calla germ on whole cucumbers fresh from the vines was tried by taking nearly ripe cucumbers, sterilizing a spot near the stem by washing with corrosive sublimate (1 part in 1,000), and then washing with sterile water. Several punctures were made in the sterilized spot with a sterile needle to the depth of from one-half to 1 inch, and two 1-mm. loops of a 24-hour-old beef-broth culture of the calla organism were applied to the sterile surface over the punctures. For control several cucumbers were treated in exactly the same manner, except that the organism was not applied. At the end of twenty-four hours at 20° to 25° C. a watery spot about one-half an inch in diameter appeared around the punctures

in the cucumbers that were inoculated. In three days from the time of inoculation the cucumbers were soft about one-half their length, and in five days they were soft throughout. The skin, however, remained intact, so that the inoculated cucumbers represented closed sacks containing a watery, pulpy mass (Pl. VI). If an opening were made in the sack the contents would flow out, leaving a semitransparent bag which could be filled with water and handled. All controls remained entirely unaffected. A drop of the watery substance from one of the inoculated cucumbers placed under a low power of the microscope showed that the cells had become separated so that each individual cell floated out by itself. The cells themselves were not collapsed, however, showing that the action of the organism had been upon the lamella connecting the cells, causing them to dissolve. This action was apparent not only upon the cucumber but upon all the raw vegetables which were rotted under the influence of this organism. The color of the cucumbers, both upon the surface and in the interior, remained unchanged. The odor of the soft contents of the inoculated cucumbers was strikingly like that arising from cucumbers that sometimes soften when pickled in brine. The reaction was distinctly acid to litmus.

To determine whether the organism that had caused the softening of the inoculated cucumbers was the calla-rot germ, a spot was sterilized on the surface of one of the soft cucumbers before the skin was broken. With a sterile needle a puncture was then made in the sterilized spot in the skin and a loop of the soft interior was removed with a sterile needle and placed in 10 c. c. of beef broth. In the usual way eight poured plates of beef agar were at once prepared from the dilutions of this beef-broth culture. In from twenty-four to forty-eight hours at 20° to 25° C. colonies appeared in all the plates. These colonies were all radiating and were alike in all respects, indicating that the cucumber contained a pure culture of an organism similar at least to the calla-rot germ. Twelve callas were inoculated with 24-hour-old beef-broth cultures made from these colonies, and in twenty-four hours the characteristic calla rot appeared in all cases, as indicated in the watery discoloration around the inoculated spots and by the subsequent decaying of the parts inoculated. In twenty-four hours more the inoculated leaves had entirely rotted off. The only part of the interior of the inoculated cucumbers not softened was the portion immediately beneath the spot sterilized for inoculation (Pl. VI, A). Here the interior remained firm, sometimes to a depth of one-half inch or more, showing that the corrosive sublimate had penetrated to a considerable depth and that the organism was unable to attack this part of the cucumber even after several days.

This series of experiments was repeated many times with practically the same results. Sometimes the action was a little slower and

sometimes a little more rapid. It was found that the action was more rapid if the cucumbers were nearly ripe before inoculation and when the temperature of the air in which they were kept after inoculation was about 30° C. Some of the experiments were carried on in the dark and some in diffused light, but there was no apparent difference in the time required for the inoculation to take, nor in the rate of progress made in softening the cucumbers in the two cases. The rate of disintegration was the same on both the upper and the lower sides of the cucumbers.

Raw green peppers.—These peppers were obtained from the market, thoroughly washed with distilled water, and afterwards with corrosive sublimate, and again rinsed with distilled water. With a sterile knife they were cut into slices and placed in sterile petri dishes, two pieces in each dish. One piece in each dish was inoculated immediately with the calla-disease organism. In twenty-four hours at 20° to 25° C. it was seen that the inoculated pieces were slightly attacked by the germ, and in forty-eight hours the disease had progressed, although not as rapidly as in the cases of the cucumber, potato, carrot, and some other vegetables. The organism attacked both the central and the outer parts of the pepper, but the change in color was not sufficient to show in a photograph even after five days. The inoculated parts were all darker than the controls (Ridgway's Parrot Green, No. 7, Plate X, or Saccardo's Atro-virens, No. 34, Table II), while the original was nearly grass green toward the outside. The interior of the pepper, originally nearly white, was changed to Cream Buff, Ridgway's No. 11, Plate V, or Saccardo's Cremeus, No. 27, Table II. The inoculated parts were also soft, had the odor of decaying peppers, and were strongly alkaline to litmus.

Raw mature onion bulbs.—The outside layers were removed and the onion was then cut into pieces of convenient thickness and placed in petri dishes, three pieces in each dish. Two of these pieces were inoculated with a 24-hour-old culture of the calla germ and one was left for control. Several dishes were prepared in this manner. The organism grew on the onion, but not rapidly, and at the end of five days at a temperature of from 20° to 25° C. the decay was apparent, although the layers of the onion were not broken down. The color was Cream Buff, No. 11, Plate V, Ridgway, or Saccardo's Cremeus, No. 27, Table II. The odor was that of decaying onions. In reaction the inoculated pieces were moderately alkaline to litmus.

Raw young onions.—Several onions were grown from seeds, and when the young plants were about two weeks old they had produced three leaves each and the longest of the leaves measured from 6 to 8 inches. These plants were inoculated with the calla organism by placing a drop of a 24-hour-old beef-broth culture on a leaf with a sterile needle and puncturing the leaf several times through the drop

of bacteria-laden broth. No sign of disease appeared in any case, although the plants were kept under observation for several weeks. This experiment was repeated several times with negative results, indicating that this organism is not a producer of disease in young green onions.

Raw pieplant.—Stalks of raw pieplant were washed with corrosive sublimate and then in distilled water. With a sterile knife the outside was removed and the stalks were then cut into slices about 2 cm. thick and four placed in each petri dish. Two of each four were inoculated with a 24-hour-old beef-broth culture of the calla germ. In two cases only was there any growth, and this was very feeble, resulting at the end of five days in a slight brown discoloration. The experiment was repeated several times, but in all cases the growth was very feeble and hardly perceptible.

Raw cabbage.—Cabbage heads were obtained from the market, the outer leaves were pulled off, and inoculations were made into the stumps and leaves of several plants, using a 24-hour-old beef-broth culture of the calla germ, several heads being left for control. In twenty-four hours the inoculated spots were slightly discolored. The color deepened for nine days (temperature, 18° to 27° C.), at the end of which time the rot had spread over the whole surface of the stumps and entirely through them. The color was Drab, No. 18, Plate III, Ridgway, or somewhat darker than Saccardo's Avellaneus, No. 7, Table I. At the same time the decay progressed in the leaves, producing the same color and advancing from leaf to leaf until at the end of nine days the whole of each inoculated head was affected. None of the control plants was affected during this time. The decayed specimens had the odor of rotten cabbage and in reaction were strongly alkaline to litmus.

In addition to these experiments with cabbage, pieces of stumps and leaves were washed with corrosive sublimate, then with sterile water, and placed in petri dishes, four pieces in each dish, two of which were immediately inoculated with a 24-hour-old beef-broth culture of the organism and two left for control. In twenty-four hours at 20° to 25° C. the inoculated pieces began to show discoloration and in five days the inoculated pieces were decayed throughout. The control pieces remained sound, except in a few instances in which the exuding juice from the decayed pieces came into contact with the controls, in which cases the latter decayed. The color, odor, and reaction were the same as in the experiments with the whole heads, as previously described.

Raw parsnips.—Raw parsnips were obtained from the market and treated in the same way as the raw potatoes. With a sterile knife pieces of convenient thickness were cut and placed in sterile petri dishes, four pieces in each dish. Two pieces in each dish were inocu-

lated with the calla-rot germ, using a 24-hour-old beef-broth culture. At the end of twenty-four hours after inoculation the inoculated pieces began to show discoloration at the points of infection, and at the end of three days (temperature, 18° to 25° C.) the discoloration was very marked (Pl. VII, fig. 1). The inoculated pieces had a pungent, sweetish odor and were plainly alkaline to litmus. The color corresponded to Ridgway's Mummy Brown, No. 10, Plate III, or nearly to Saccardo's Fuligineus, No. 11, Table I.

Raw carrots.—Several roots of carrots were obtained from the market and prepared in the manner indicated above. Slices of suitable thickness to be placed in petri dishes were then cut off with a sterile knife. Four pieces were placed in each petri dish, and as in the other experiments two out of each set were inoculated with the calla-rot organism and two left for control. In twenty-four hours at 20° to 22° C. the inoculated pieces began to discolor at the points of inoculation, and in three days the discoloration was very striking over the entire surface of the inoculated pieces (Pl. VII, fig. 2). In the central part of the root the discoloration had extended entirely through, a distance of 2 cm., while toward the outer surface the progress was not so rapid, the discoloration having extended only about 1 cm. The color of the inoculated pieces three days after inoculation was Vandyke Brown, No. 5, Ridgway's Plate III, or nearly Saccardo's Fuligineus, No. 11, Table I. The decayed part was distinctly alkaline to litmus. At the end of eight days the inoculated pieces were entirely discolored and soft, while the uninoculated pieces still retained their normal color and were sound. At this time the inoculated pieces had changed in color from Vandyke Brown or Fuligineus to Olive, No. 9, Ridgway's Plate III, or to Saccardo's Olivaceus, No. 39, Table II.

Raw turnips.—A firm, white turnip was obtained from the market, prepared for the petri dishes, and inoculated in the same manner as the other vegetables. In twenty-four hours discoloration was distinctly noticeable at the points of inoculation, and in three days the discoloration was very striking and had progressed downward from 2 to 3 mm., while the uninoculated pieces were still white and sound (see Pl. VIII, fig. 1). The color of the inoculated pieces at this time closely resembled Ridgway's Olive, No. 9, Plate III, or Saccardo's Olivaceus, No. 39, Table II. The discolored parts were strongly alkaline to litmus and had a striking odor of decayed turnips.

Raw salsify.—Several roots of salsify were obtained from the market and the same method was used in preparing and inoculating them that was employed with the other vegetables. In twenty-four hours the inoculated pieces were discolored and in three days all had discolored but only the inoculated pieces had decayed, and as these kept their shape it was impossible to bring out the difference in color by

means of a photograph. The growth of the organism, however, was apparently just as rapid in the salsify as in the parsnips, carrots, etc. The inoculated pieces were alkaline to litmus and had an odor of decaying salsify.

Raw tomatoes, ripe.—Several ripe tomatoes were inoculated with a 24-hour-old beef-broth culture of the calla germ. Before inoculating, a spot about one inch in diameter on the surface of the fruit was washed with a dilute solution of corrosive sublimate and then with sterile water. A loop of the culture was then placed on the sterilized spot and a sterile needle was used to puncture the skin through the drop of beef-broth culture. Some of the tomatoes so inoculated were left in diffused light, some were placed in a dark room, and all were maintained at a temperature of about 18° C. Twenty-four hours after inoculation each infected spot was surrounded by a watery area about 1¼ inches in diameter. The contents of the inoculated tomatoes softened rapidly, so that at the end of four days after inoculation openings were made in the skins of some of the infected fruits and the contents were poured out, leaving the skins intact. The cell contents of the inoculated tomatoes were apparently acted upon by some substance that dissolved the intercellular layers and allowed the individual cells to become entirely separated, as in the case of the cucumbers already cited. The cell contents did not seem to be affected, nor did the substance act upon the skin of the tomato.

Raw tomatoes, green.—Some tomato plants growing in the Department greenhouse bore a number of unripe tomatoes varying from 1 to 2 inches in diameter. Six of these were inoculated on the plants in the same manner as the ripe tomatoes described above. Twenty-four hours after inoculation (temperature, about 30° C.) all the infected tomatoes had small watery spots at the point of inoculation. Twenty-four hours later the watery spots appeared sunken and whitish. In another twenty-four hours the spots began to turn brown, the skin cracked, and the juice began to ooze out. In twelve days after inoculation the contents had oozed from all the inoculated tomatoes, leaving the skins still clinging to the vines. Plate VIII, figure 2, shows a photograph of one of the skins (No. 2) and of an uninoculated tomato (No. 1) on a piece of one of the vines. The skins did not cling firmly to the vines, but could be easily removed. The stems to which the skins were attached had a discolored and dead appearance, but were not at all soft. Green tomatoes brought into contact, either artificially or naturally, with a decayed tomato did not take the disease. While the general effect of the organism is the same upon the green as upon the ripe tomato, the progress is much more rapid in the case of the ripe fruits.

Raw apples (York Imperial).—The outside of the apple was washed with corrosive sublimate (1 part in 1,000) and then with sterile water.

Several pieces were then cut out with a sterile knife and placed in sterile petri dishes, four pieces in each dish. Two pieces in each dish were inoculated with a 24-hour-old culture of the calla-rot germ in beef broth and two pieces were left for control. After four days a slight growth was noticeable, but the rate of growth was very slow.

Raw pineapples.—The outside was removed and several pieces were cut from the interior with a sterile knife. As in the previous case, four pieces were placed in each of several petri dishes. Two pieces in each dish were inoculated as above and two left for control. These preparations were kept for about ten days, but no growth appeared on any of the pieces.

Raw yellow bananas.—The outside of the bananas was carefully peeled off, and with a sterile knife cross sections from 1½ to 2 cm. thick were cut off and placed in sterile petri dishes, four in each dish. As in the preceding cases, two pieces in each were inoculated with a 24-hour-old culture of the calla-rot germ in beef broth and two were left for control. After ten days no growth was noticeable on any of the pieces.

GAS.

To determine whether or not the calla-rot organism is a gas producer, six solutions were used, viz, peptone water +1 per cent mannite, peptone water +1 per cent maltose, peptone water +1 per cent dextrose, peptone water +1 per cent cane sugar, peptone water +1 per cent milk sugar, and peptone water +1 per cent glycerin. A half dozen fermentation tubes were filled with each of these solutions, and after sterilizing for fifteen minutes on three consecutive days several tubes of each set were inoculated with a 1-mm. loop of a 24-hour-old beef-broth culture of the calla-rot organism. A part of each set was left for control. In eighteen hours after inoculation of the infected tubes (temperature, 20° C.) they were clouded in the bulb, and the clouding extended from one-half to 1 inch into the closed ends of the tubes. In forty hours the clouding extended to the top of the closed end of each inoculated tube, but no gas had formed in any case. (Fig. 6.) The control tubes were all clear and free from gas. These tubes were kept under observation for two weeks, but no gas formed in any of the tubes, and the control tubes were still clear and free from sediment. The inoculated peptone-mannite tubes began to clear at the top of the closed ends in from twenty to thirty weeks after inoculation. The deposit formed from a settling of the sediment was cream buff in color, as seen by reflected light, and corresponded very nearly to Ridgway's No. 11, Plate V. The reaction of the contents of the tube was slightly acid to litmus at the close of the experiment. The inoculated peptone-maltose tubes began to clear in from ten to twelve weeks, and by the end of twenty weeks were entirely clear. The

deposit formed was only about one-half the bulk of the deposit in the peptone-mannite tubes. It was of a drab color, corresponding very closely to Ridgway's Ecru Drab, or a little darker than Saccardo's Avellaneus, No. 7, Table I, when viewed by reflected light. The reaction of the contents of the tubes was slightly alkaline to litmus at the close of the experiment. The peptone-dextrose tubes began to clear in from ten to twelve weeks after inoculation, and in twenty weeks were entirely clear. A large part of the sediment clung to the back of the upright part of the tube instead of settling completely, as in the other inoculated tubes. The color of the deposit was also drab, corresponding very closely to Ridgway's Ecru Drab, No. 21, Plate III, or a little darker than Saccardo's Avellaneus, No. 7, Table I, when seen by reflected light. The reaction of the contents of the tube at the close of the experiment was slightly acid to litmus. The cane sugar, milk sugar, and glycerin tubes cleared in from one to six weeks. The glycerin tube cleared first, then the milk-sugar tube, and lastly the cane-sugar tubes. The deposit was heaviest—about 4 mm. deep—in the cane-sugar tubes, about 2 mm. deep in the milk-sugar tubes, and only 1 mm. deep in the glycerin tube. The color of the deposit was the same as in the other cases, viz, Ridgway's Ecru Drab, No. 21, Plate III, or a little darker than Saccardo's Avellaneus, No. 7, Table I. Each inoculated tube gave an acid reaction with litmus at the close of the experiment. No gas formed in any of the tubes. It is therefore apparent that the calla-rot organism is not capable of splitting up mannite, maltose, dextrose, cane sugar, milk sugar, or glycerin so that a gas will form.

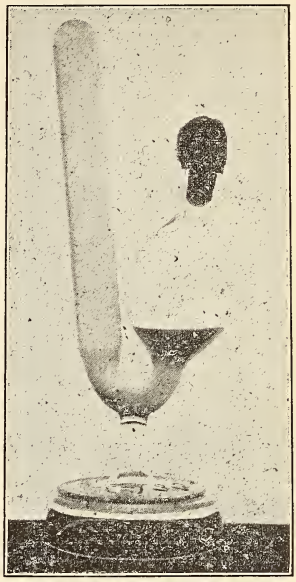

FIG. 6.—Fermentation tube ten days after inoculating with the calla organism.

ACTION ON LEAD ACETATE.

Slant tubes of lactose agar, colored with litmus, were inoculated with the calla-rot organism, and at the same time slips of filter paper saturated with lead acetate were introduced into the tubes. These paper strips were held at one end by a cotton plug, so that they did not come into contact with the medium. In twenty-four hours the color began to fade from the litmus-lactose agar, and in three days the agar was practically colorless, except a small area near the top, which was still slightly tinged. At the same time the lead acetate paper began to blacken around the edges. Twenty-four hours later the margins of the paper strips were still darker and the discoloration

extended a little farther from the edge. At the end of eight days from the beginning of the experiment the color had entirely disappeared from the inoculated tubes, while it remained unchanged in the controls. The lead-acetate papers were blackened about three-fourths of an inch from the lower end upward, the color fading out and leaving no sharp line of demarcation. The liquid that settled in the angle of the inoculated tubes at the end of eight days had become nearly cream color, corresponding closely to Ridgway's No. 20, Plate VI, or Saccardo's Cremeus, No. 27, Table II, while in the control tubes the liquid was still litmus color. At the expiration of twenty-seven days from the beginning of the experiment the color began to return in the agar, and seven days later the original color had returned throughout the agar and also in the liquid that had previously been cream color. As soon as the color began to return to the agar the discoloration of the lead-acetate slips ceased to develop. The black color in the lead-acetate papers was undoubtedly due to the formation of hydrogen sulphid, which develops on certain media during the activity of the calla-rot organism. As soon as the organism became inactive the hydrogen sulphid ceased to form, and what had formed passed off from the agar, allowing the litmus color to return. Beef broth inoculated with the calla-rot organism discolored the margins of lead-acetate paper in twenty-four hours, the discoloration extending about one-fourth of an inch from the margin. This gas forms much more rapidly in beef broth than in litmus-lactose agar, while the organism growing on potato cylinders produced no blackening of lead-acetate strips, even at the end of three weeks after inoculation.

INDOL.

Several tubes of peptonized Uschinsky's solution were inoculated with fresh cultures of the calla-rot organism. The inoculated tubes clouded within twenty-four hours, and tests were made from day to day for indol, using concentrated sulphuric acid and sodium nitrite, but even at the end of twenty-four days no trace of indol could be detected, although the tubes were heated to 80° C. after the application of the acid and the nitrite.

NITRATES REDUCED TO NITRITES.

Four tubes of nitrate bouillon were inoculated with the calla germ. These became distinctly clouded in the usual time, and at the end of two days were tested for nitrites as follows: To 10 c. c. of the clouded bouillion 1 c. c. of starch solution and 1 c. c. of potassium iodid solution were added. One drop of sulphuric acid was then sufficient to give an intensely blue color, indicating that the nitrates had been changed to nitrites. The control tubes treated in the same manner gave no reaction.

MAXIMUM TEMPERATURE.

In determining the maximum temperature at which the calla-rot organism will grow several media were used, viz, agar, gelatin, beef broth, and Uschinsky's solution. These media were inoculated with a 24-hour-old culture of the calla-rot organism in beef broth, and several tubes of each medium were placed in an incubator which registered 40° C. At the expiration of forty-eight hours the temperature still remained at 40° C., and there was no visible growth in any of the media. Growth was apparent in all the control tubes at the end of twenty-four hours after inoculation. On the third day after the tubes were placed in the oven the temperature fell to 38° C., and at the expiration of twenty-four hours thereafter there was a visible clouding of the beef broth and of the Uschinsky solution, but no growth appeared on the other media. When the incubator had again become steady at 40° C., fresh cultures were introduced, including, in addition to the above mentioned media, milk, litmus milk, and poured-agar plates. At the end of forty-eight hours there was a slight clouding of the beef broth and of the Uschinsky solution, but no growth was yet apparent in the other media. Twenty-four hours later the clouding in the beef broth and in Uschinsky's solution had increased and minute colonies began to appear in the poured plates, slight growth being apparent also on slant agar and stab gelatin cultures. At the end of another twenty-four hours the milk was slightly curdled and the litmus milk was beginning to redden. The temperature remained constantly at 40° C., and growth advanced slowly in all cases for several days. The colonies in the poured plates increased in size until they were from 2 to 3 mm. in diameter. It should be noted that all the colonies produced on the agar plates at this high temperature were round, none of them showing any tendency to radiate as they did under temperatures from 20° to 30° C. While 40° C. retards the growth of the organism it does not prevent it. The incubator was next regulated at 41° C. and fresh cultures of the organism on the various media were placed in it. After forty-eight hours there was a slight growth in the Uschinsky solution and on the slant agar, but it was very slight as compared with the controls. No growth appeared in the other media. At the end of another forty-eight hours, growth in the agar and in the Uschinsky solution was not perceptibly advanced and no growth appeared in any of the other media. Upon removing all these cultures to conditions of normal temperature at the end of the fourth day, growth advanced rapidly in those cases where it had started and appeared in all the other media used within twenty-four hours after removal. When fresh cultures were kept constantly at 42° C. no growth appeared, but exposure to this temperature for twenty-four hours did not destroy the life of the organism, as evidenced

by the fact that when the cultures were removed from the incubator at 42° and kept at 20° C. growth began within a few hours. If fresh cultures were placed in the incubator at 43° C. life was not destroyed within fifteen hours, but cultures removed at the end of twenty-four hours and placed under normal conditions failed to grow. If the temperature was kept constantly above 41° C. no growth appeared in any of the media used. Hence after many repeated tests it was decided that 41° C. is the maximum temperature at which this organism will grow.

MINIMUM TEMPERATURE.

To determine the lowest temperature at which the calla-rot organism will grow, fresh cultures were placed in the ice box at different elevations, with as little variation as possible in the quantity of ice, so that the temperature remained fairly constant for each set of cultures, but varied for the different sets from about 3° to 9° C. Set 1 consisted of cultures of beef broth, Uschinsky's solution, gelatin stab cultures, and slant agar, and was kept at a temperature between 3° and 5° C. for twenty-four days. The control cultures at room temperatures of 20° C. produced growth as usual within twenty-four hours, while the cultures at the low temperature showed no signs of growth until they were removed from the ice box at the expiration of twenty-four days, when all produced growth within twenty-four hours. Set 2 was kept at approximately 6° C. for nine days, at the end of which time growth appeared, slightly clouding the beef broth. The temperature sometimes fell to 5° C., but did not at any time during the nine days exceed 6½° C. Set 3 was kept at approximately 9° C. Slight growth began in from two to four days. Beef broth was the first to show the growth in the low temperatures, while in the high temperatures it was usually the Uschinsky solution that clouded first. Six and one-half degrees centigrade seems to be the lowest temperature at which growth will take place. At 9° C. growth takes place slowly and the colonies in agar-plate cultures at this temperature are small and round, as was found to be the case in the high temperatures.

OPTIMUM TEMPERATURE.

The calla-rot organism grows readily between 15° and 37° C. Fresh cultures of beef broth, Uschinsky's solution, and agar inoculated with a 1 mm. loop of a 24-hour-old beef-broth culture, placed in an incubator at 37.5° C., showed signs of growth within six hours. Similar cultures at 35° C. showed a distinct growth in four hours. As it is sometimes difficult to compare culture solutions accurately with reference to the intensity of clouding, agar-plate cultures were also used. The fresh cultures were placed at different temperatures—some at 20°, some at 30°, some at 33°, some at 35°, and some at 37.5° C. In fifteen hours the plates at 35° C. showed the colonies most distinctly.

The colonies measured from 1 to 3 mm. in diameter. Colonies were also visible in the plates at 20° and 30° and at 37.5° C., but they were smaller—scarcely larger than pin points. Similar tests were made of other temperatures above and below 35° C. with like results. Since all growth above and below 35° C. is slower than at this temperature, it appears that 35° C. is the optimum temperature for the growth of the calla-rot organism. In thirty-four hours the colonies at 35° C. had the characteristic radiating form, while those at and above 37.5° C. were round.

THERMAL DEATH POINT.

The thermal death point is the lowest temperature at which the life of the organism will be destroyed when a fresh culture is exposed to that temperature for ten minutes. To determine that point with the calla-rot organism fresh beef-broth cultures were made from a 24-hour-old culture of beef broth, each culture consisting of 10 c. c. of broth inoculated with a 1-mm. loop of the 24-hour-old culture. The tubes containing these fresh cultures were placed in water at constant temperature for ten minutes. In the first experiment three sets of tubes were used. One set was exposed to a temperature of 49°, another set was exposed to 49.20°, and the third set was exposed to 49.40° C. After exposing the tubes to these temperatures they were placed at room temperature of about 20° C., and at the expiration of eighteen hours all control tubes were clouded and all exposed tubes were clear. Six hours later set 1 (49° C.) was clouded slightly; sets 2 and 3 were still clear. Twenty-four hours later—i. e., forty-eight hours from the time the tubes were exposed to the heat—all inoculated tubes were clouded. In the second experiment three sets of tubes were again used. After inoculating in the same manner as above, one set was exposed for 10 minutes to a temperature of 49.50°, another to 50°, and a third to 50.20° C. Several inoculated tubes were left untreated for control. At the expiration of twenty-four hours all control tubes were clouded, and all exposed tubes were clear. Twenty-four hours later four tubes in set 1 (49.50° C.) were clouded and two were clear. All tubes in sets 2 and 3 (12 in all) were still clear. At the expiration of two weeks all tubes in sets 2 and 3 were still clear, and the two tubes in set 1 were also clear. Agar plates were made from the clouded tubes that were heated to 49.50° C., and in all cases pure cultures of the calla organism were obtained, as indicated by the shape of the colony and by the fact that inoculations into calla plants produced the characteristic symptoms of the disease. Several sets of cultures were subsequently exposed to a temperature of 50° C. for ten minutes, but always with the result that they all remained clear indefinitely, while a part, at least, of the cultures exposed below 50° C. clouded in a longer or shorter time, showing that 50° C. is the thermal death point for this organism.

DIFFUSED LIGHT.

Diffused light had no effect upon the development of the organism in any of the media used, i. e., beef broth and other liquid media, clouded or otherwise, showed the presence of the organism as readily under one condition as the other, and in the agar plates the colonies formed as quickly and grew as rapidly in diffused light as in the dark.

DIRECT SUNLIGHT.

To determine the effect of direct sunlight upon the organism several tubes, each containing 10 c. c. of agar, were inoculated and poured into thin petri dishes. One-half of each dish was covered with black paper and the dishes were then exposed to the direct sunlight. Some of the dishes were removed from the direct sunlight at the end of five, ten, fifteen, twenty, and sixty minutes. In those dishes that were exposed five minutes only, colonies appeared in all points of the plate in twenty hours. The colonies appeared just as readily and grew just as rapidly in the exposed as in the unexposed part of the plate, but were a little less numerous, showing that a few of the organisms had been killed by the direct light in five minutes. In the plates that were exposed ten minutes colonies appeared in the covered part of the plate within twenty-four hours, but none appeared in the exposed part of the plate until nearly forty-eight hours after being placed in diffused light. The colonies which finally formed in the exposed part were much less numerous than those in the shaded part. In the covered part of the plate that was exposed fifteen minutes colonies appeared within twenty hours, but no colonies appeared in the exposed side, even at the end of a week, except a few around the edge of the plate, which were apparently protected slightly either by the shadow of the margin of the petri dish or by the organism being several deep around the margin of the plate, so that the upper layers protected those below from being destroyed by the direct rays of the sun. The same was true of the plates exposed twenty minutes. It appears, therefore, that from five to fifteen minutes of direct sunlight are sufficient to destroy the life of the organism, but that a very slight protection only is necessary to prevent them from being destroyed. Even in the plates exposed for sixty minutes the organisms around the margin of the plate were likewise protected. In all cases colonies appeared close to the dividing line between the exposed and the shaded part of the plate, and growth extended in every instance from these marginal colonies into the exposed part of the plate, showing the characteristic radiation of the colonies when not crowded.

EFFECT OF NITROGEN.

Several tubes of beef broth were inoculated with the calla-rot germ and the tubes were placed immediately in a jar from which the oxygen

was removed by the aid of pyrogallic acid and sodium hydrate, thus leaving practically an atmosphere of nitrogen. The jar was placed in diffused light at a temperature of from 18° to 25° C. At the expiration of thirty-five days it was opened and the beef broth was as clear as if it had not been inoculated, showing that no growth had taken place in the absence of oxygen. Twenty-four hours after the jar was opened the tubes were clouded as deeply as if the inoculation had been made the day the jar was opened instead of thirty-five days prior to that time. Hence, while nitrogen will not enable the organism to grow, its life is not destroyed by the action of this gas, and when inoculations were made from these cultures into callas the disease promptly appeared, and in forty-eight hours the inoculated leaves and flower stalks had rotted off. Agar-poured plates made from the clouded tubes and from the diseased portion of the inoculated calla showed the same characteristic pure cultures composed of radiating colonies. To determine how much longer the organism would live in the absence of oxygen, cotton-plugged tubes of beef broth, Uschinsky's solution, and a mixture of Dunham's and Uschinsky's solutions (half and half) were inoculated with the calla organism and were kept in an atmosphere of nitrogen two hundred and seventy-five days, in the manner described above. At the expiration of this time the tubes, all of which were clear, were exposed to the air at room temperature, i. e., 18° to 25° C., the same temperature at which they had been kept in the atmosphere free from oxygen. The atmosphere in the jar would not support combustion at the moment it was opened, indicating that the oxygen had not diffused into it. In twenty-four hours after exposing the tubes to the air the Uschinsky solution and the mixture of the Uschinsky and Dunham solutions were all clouded, but the beef-broth solutions were not clouded. The clouding increased for several days in those tubes in which it had begun, but no growth appeared in the beef broth even after several weeks of exposure to the air. Poured plates and inoculations into healthy callas from the clouded tubes showed that this was the calla organism.

EFFECT OF CARBON DIOXID.

Freshly inoculated tubes of slant agar, Uschinsky's solution, nitrate bouillon, and common bouillon were placed in an air-tight jar into which carbon dioxid was passed. Before the gas entered the jar containing the tubes it was passed through solutions of potassium permanganate, sodium hydrate, and distilled water. After being filled and exhausted six times, to insure an atmosphere of pure carbon dioxid, the jar was filled with the gas, sealed, and allowed to stand for fourteen days. At the expiration of this time it was opened and the tubes were examined. The slant agar showed a thin, pure white growth the whole length of the streak and a small amount of whitish precipitate in the fluid in the angle formed by the agar and the side of the

tube. The amount of growth was only moderate. The Uschinsky's solution showed no growth at this time. In twenty-four hours the tubes of Uschinsky's solution were still clear, but at the end of forty-eight hours after exposure to the air the solution was distinctly clouded, showing that free oxygen is necessary for the growth of the calla organism in Uschinsky's solution.

In the nitrate bouillon there was only a moderate amount of growth at the time the jar was opened, but the solution was distinctly clouded. There was a white precipitate 7 mm. in breadth, but no pellicle or rim had formed. The nitrates were reduced to nitrites, as shown by the usual test. The common bouillon was distinctly and uniformly clouded. Apparently the growth had been twice as rapid as in the nitrate bouillon, as indicated by the degree of cloudiness of the tubes and by the large amount of white precipitate, which was fully twice as abundant as in the nitrate bouillon tubes. No rim or pellicle formed in any of the tubes.

EFFECT OF HYDROGEN.

Tubes of slant agar, Uschinsky's solution, ordinary bouillon, and nitrate bouillon were inoculated with the calla organism and placed in a hydrogen atmosphere. The hydrogen was generated by the action of dilute sulphuric acid upon zinc. The gas thus produced was passed through solutions of silver nitrate, potassium permanganate, sodium hydrate, and distilled water into a chamber containing the inoculated tubes. The chamber was filled and exhausted six times, thus insuring practically a pure atmosphere of hydrogen. The chamber was then sealed and left undisturbed for twenty days, at the end of which time the following results were noted:

The organism had made a feeble growth on the slant agar, as indicated by a very faint streak along the surface of the medium, and a small amount of whitish precipitate to the depth of 2 mm. had been deposited in the angle between the agar and the side of the tube. Uschinsky's solution was feebly clouded throughout. A small amount of deposit to the breadth of 7 to 8 mm. had formed in the bottom of the tube. The ordinary bouillon was feebly clouded throughout and a white precipitate 8 mm. in breadth had been deposited. The nitrate bouillon was feebly clouded, with a small amount of white deposit 12 mm. broad in the bottom of the tube. No rim or pellicle had formed in any of the fluids.

COMPARISON OF CALLA-ROT GERM WITH SIMILAR ORGANISMS.

Bacillus carotovorus Jones.[a]—Upon comparing the calla organism with the carrot-rot germ, as described by Jones, it is found to differ in

[a] Jones, L. R. A Soft Rot of Carrot and Other Vegetables Caused by Bacillus Carotovorus, Jones. Thirteenth Annual Report of the Vermont Experiment Station, 1900, p. 299.

several particulars—i. e., the calla rot does not, while the latter does produce gas. The former is not affected by diffused light, while the latter is affected, etc. The shape of colonies differs. There are, of course, numerous points in which the two organisms agree, but they differ in enough essential points to show that they are not the same.

Bacillus oleraceæ Harrison.[a]—Cultures of this organism were obtained, and repeated inoculations were made with fresh cultures into various parts of calla plants. At the same time parallel inoculations were made with similar cultures of the calla-rot germ. In twenty-four hours after inoculation nearly all the plants inoculated with the calla germ showed the characteristic symptoms of disease, and the decay continued to progress until the plants were practically destroyed. On the other hand Harrison's organism did not affect the plants in any way, showing that the two organisms are not identical.

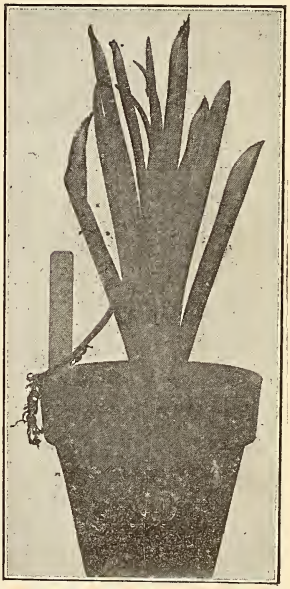

FIG. 7.—Hothouse hyacinth inoculated in a flower with the calla organism.

Heinz's hyacinth germ (*Bacillus hyacinthi septicus*).[b]—In order to learn the effect of the calla organism on hyacinths, more than 100 hyacinths were inoculated with fresh cultures of the calla germ. The leaves, flower stalk, and flowers were inoculated. Most of the inoculations were made in plants growing in the open when the weather was bright and warm. A few hyacinths were potted and placed in a greenhouse. The flowers were inoculated by dropping a single drop of a 24-hour-old beef-broth culture into the flower. The leaves and flower stalks were inoculated by scraping a quantity of the fresh growth of the organism from a slant-agar surface, applying it to the diseased spot, and then puncturing the plant with a sterile needle through the mass of organisms. None of the plants in the open showed any symptoms of the disease whatever, although they were watched daily for more than two weeks. The inoculated plants in the greenhouse did not show any symptoms of disease until the expiration of five days, when a few of the leaves and flower stalks began to soften. The affected parts gradually decayed throughout (fig. 7). Pure cultures of the calla organism were obtained from these diseased parts of the hyacinths. The difficulty with which this organism

[a] Harrison, F. C. Preliminary Note on a New Organism Producing Rot in Cauliflower and Allied Plants. Science, n. s., Vol. XVI, July 25, 1902, p. 152.

[b] Heinz, A. Zur Kenntniss der Rotzkrankheiten der Pflanzen. Centralblatt f. Bakt. u. Parasitenkunde, Bd. V, 1889, p. 535.

affects the hyacinths indicates that it is not the same as Heinz's hyacinth germ, which attacked the plants readily and destroyed them rapidly when inoculated by either of the methods used in these tests. Heinz's organism (*Bacillus hyacinthi septicus*) does not liquefy gelatin, while the opposite is true of the calla organism. The colonies in plate cultures are round and when grown on sterile potato they are a dirty yellow color. The colonies of the calla organism are usually radiating and on potato they produce a brownish color.

Potter's Pseudomonas destructans.[a]—Potter's organism, when grown in a solution containing sugar, liberates carbonic acid gas. The calla organism is not a gas producer. Colonies in plate cultures are round, and when grown on vegetables the end reaction is acid. The calla organism usually produces radiating colonies, and on vegetables the end reaction is generally alkaline. *Pseudomonas destructans* has but one flagellum while the calla organism has several flagella.

Likewise in comparison with other forms the calla germ does not agree in all particulars with any other known organism, and the writer therefore proposes for the calla-rot germ the name *Bacillus aroideæ.*

ORIGIN AND SPREAD OF THE DISEASE.

The calla rot has been reported from the Western, Central, and Eastern States, i. e., from the Atlantic to the Pacific. It therefore appears to have spread over the entire calla-growing section of the United States, but it is much more destructive in some portions of the country than in others. It causes a loss of thousands of dollars annually and has become so destructive in some sections that the growers have either abandoned the calla altogether or have greatly reduced the space and time that they have heretofore devoted to this plant. It is therefore of the highest importance that the grower should know the source of this disease and the ways in which it may spread from place to place and from plant to plant.

Calla corms that are attacked late in the season go into their resting stage in a partly decayed condition. If the attack has been slight the infected spot will dry down and may be overlooked when corms are selected the following season for growing calla plants. When callas begin to grow from such corms the organisms which have remained dormant during the resting period of the corm are revived and decay is started afresh. Since this organism may remain dormant for months without its life becoming extinct, it may be spread from one locality to another, and even from country to country, whenever diseased corms are transported. It is undoubtedly in this manner that the disease has become so widespread in this country.

[a] Potter, M. C. Ueber eine Bakterienkrankheit der Ruben. Centralblatt f. Bakt. u. Parasitenkunde, Bd. VII, II. Abt., 1901, pp. 282, 353.

The spread of the disease from plant to plant in the same house seems to be accomplished mainly through the soil. One reaches this conclusion from the fact that healthy calla plants growing in pots and standing near diseased callas are less likely to become infected than when similar healthy plants are growing in a solid bed with diseased corms. Furthermore, it is almost always the case that the disease, if undisturbed, first attacks the corm beneath or just at the surface of the ground.

Usually the first season that the disease appears only a few of the plants are actually destroyed, but the millions of organisms which are produced during the process of decay remain in the soil, and some of them reach corms that were perfectly healthy when planted. These infections, as already indicated, often produce the hold-over cases, which develop the following season. The organism may be carried from plant to plant by stirring the soil after some of the corms have become well rotted, or simply by walking about on the bed in cutting the flowers.

The nature of the soil apparently has much to do with the spread of the disease in the bed. A soil that is rich in vegetable matter is a better medium for the organism to grow and spread in than a soil that is poor in such material. Furthermore, a soil filled with humus retains the moisture better than one that is lacking in vegetable matter, a condition that greatly aids the multiplication of the organism. It often happens that the roots reach from corm to corm through the soil of the solid bed. Usually the corms are placed about 12 inches apart each way, and it is not uncommon for the plants to produce roots from 6 to 12 inches in length. Plate IX shows a small plant with a root more than 6 inches long. The writer has frequently been able to follow the progress of the disease through these roots from plant to plant. The contents of a calla root affected with this disease become soft, while the epidermis remains intact. The diseased roots are also somewhat darker than the healthy ones, so that they can be distinguished readily by sight as well as by touch. These appear to be the principal methods by which this disease is spread from plant to plant in the solid bed.

The only insect that has been observed by the writer in connection with the diseased plants is the so-called bulb-mite, but in no case has this insect been found on any part of a healthy plant and only on the decayed part of the diseased plants. To determine whether or not those insects were at all responsible for the spread of the disease a large number of mites were placed in petri dishes containing pure cultures of the calla organism. After the mites had come into contact with the colonies of bacteria they were transferred to healthy callas. Some were placed on the corms, others on the leaves, and still others on the flower stalks, but in no case did any of these plants develop the rot.

REMEDIES.

Various methods have been used with the hope of finding some remedy by which the progress of the disease could be stopped after the plants became infected. With this end in view the following treatments were used: The partly decayed corms were treated with the following substances, viz, air-slaked lime (two parts of the same with one part sulphur), formalin (varying from 1 to 10 per cent), corrosive sublimate, Bordeaux mixture, and copper sulphate solution. These were used on plants in different stages of decay. In some cases the soft part of the bulb was scraped away with a clean knife before the substance was applied, and in other instances the material was placed on the decayed part without in any way disturbing it. Sometimes the softened part was scraped away and nothing was applied, simply leaving the exposed surface to dry down. None of the treatments, however, was entirely successful. The lime and the lime and sulphur retarded the progress of the disease, but in a few cases only did the disease seem to be entirely eradicated. The solutions used appeared to make no impression upon the disease unless they were of sufficient strength to kill the plant. A few of the plants that were scraped and left without further treatment did not suffer further decay, but the percentage of cases of this kind was very low.

The successful treatment of the diseased plants in the bed was considered impracticable, and preventive measures were then resorted to. The soil was all removed from the solid bed in which practically all the callas had decayed, and this was replaced with fresh soil. At the proper time a new set of corms was obtained, but they were not planted directly in the bed. They were first carefully examined and all that showed suspicious dark-colored spots were discarded. The remainder were started in pots and then transplanted. This made it possible to discard all plants which showed any indication of the rot after growth began. As a result no disease appeared in the bed of 1,000 callas during the entire season. The same soil was used the second and third years and the same precautions were taken in regard to putting into the bed only healthy bulbs, so far as possible, with the result that while a few diseased plants appeared successful crops of callas were grown. Plate I shows the third consecutive lot of callas in the same bed since the crop was entirely destroyed by the soft rot. Very little of the disease has appeared owing to the precautions that were taken in changing the soil and in selecting healthy corms.

It is safe, therefore, to state that the soft rot of the calla may be prevented or held in check sufficiently for all practical purposes by changing the soil every third or fourth year, depending upon the number of cases of rot that appear, and by exercising due caution in selecting only healthy plants for the bed. Diseased corms may often

be detected, even in the dormant state, by examining for discolored spots, but it is safer to start the plants in pots, even after the corms having discolored areas have been rejected, to insure getting as few diseased plants as possible in the bed, since experience shows that some corms are so slightly affected that the disease is not easily detected in the dormant state. Some growers prefer to keep their plants in pots throughout the season as a preventive measure against the rot, but as a rule callas grown in this manner do not produce as large flowers as when grown in a solid bed. Hence, if the trade demands a large flower, the solid bed is preferable.

In conclusion, the writer wishes to express his acknowledgment to Dr. Erwin F. Smith, pathologist in charge of the laboratory of plant pathology, for his many helpful suggestions and his assistance in carrying on this work, and also to Mr. Alexander B. Garden, of Anacostia, D. C., for his kindness in allowing free access to his calla house during the past four years.

SUMMARY.

(1) The soft rot of the calla is a bacterial disease.

(2) The organism that produces the calla rot is a short rod bearing peritrichiate flagella.

(3) The organism occupies the intercellular space in its host and dissolves the layers that connect the cells, causing the affected tissue to break down into a soft, slimy mass.

(4) The organism is able to attack a large number of raw vegetables, and is capable of producing soft rot in many of our useful plants. Care should therefore be taken not to throw any decayed or partly decayed callas or the soil from a bed in which callas have decayed in any place where the vegetables mentioned in this bulletin are to be grown.

(5) It does not attack tree fruits readily, and hence is not likely to produce fruit rots.

(6) It grows readily on beef agar, forming at room temperature (18° to 25° C.) radiating colonies, while on the same medium at extreme temperatures (8° or 37°) the colonies are usually round.

(7) It liquefies gelatin.

(8) It coagulates milk, and first reddens, then bleaches blue litmus milk.

(9) A 1-mm. loop of a fresh fluid culture of the organism placed in 10 c. c. of beef broth will distinctly cloud it in four hours at 35° C.

(10) The organism does not produce gas when grown in a peptone solution containing 1 per cent of cane sugar, milk sugar, glycerin, maltose, dextrose, or mannite.

(11) It bleaches litmus lactose agar.

(12) It will not grow at a temperature below 6° C., nor at a temperature above 41° C., and grows best at 35° C.

(13) The life of the organism is destroyed if it is kept for ten minutes in tubes of beef broth at or above 50° C.

(14) Its growth is not affected by diffused light, but direct sunlight will kill the organism in from five to fifteen minutes.

(15) It will not grow in an atmosphere from which the oxygen has been removed, but will remain alive for many months in this condition at a room temperature of 18° to 25° C.

(16) It does not grow well in an atmosphere of pure hydrogen.

(17) Its growth is very slight in an atmosphere of carbon dioxid.

(18) When grown on vegetables the end reaction is usually alkaline to litmus.

(19) The organism may remain dormant for many months in partly decayed corms, a condition which enables the disease to be transported long distances and to be held over from year to year.

(20) The soft rot of the calla may be prevented by a careful selection of sound corms and by changing the soil in the calla beds at intervals of three or four years.

(21) Brief description of the organism:

B. aroideæ n. sp. A short rod with rounded ends, generally single or in doublets or 4's, but under certain conditions growing in chains. Usual length when taken from a beef-broth culture 24 hours old 2μ–3μ, breadth about 0.5μ and fairly constant. Organism motile, flagella 2 to 8, peritrichiate. Growth white or nearly so on the various solid media. Aerobic and facultative anærobic. Not a gas producer. Liquefies gelatin; reddens litmus milk, separates the casein from the whey and solidifies the former. Grows slowly on potato cylinders, where it is white with a tinge of yellow, the potato being distinctly grayed. Growth good and vitality long in Uschinsky's solution. No indol produced. Nitrates reduced to nitrites. Methylene blue in Dunham's solution is changed to green on addition of grape sugar. Does not grow in nitrogen but remains alive many months. Grows feebly in hydrogen and carbon dioxid. Minimum temperature for growth about 6° C.; optimum, 35° C.; maximum, 41° C., thermal death point, 50° C. Surface colonies on agar, round at temperatures near the maximum and minimum, but fimbriate at optimum temperature.

B. aroideæ was isolated from rotting calla corms and is the cause of a soft rot of the corm, petiole, and flower stalk of the calla lily. It also causes a soft, dark colored rot when inoculated into many raw vegetables, such as carrot, potato, turnip, radish, cabbage, and cauliflower. It also causes a soft rot of certain green fruits, such as the tomato, eggplant, and cucumber.

PLATES.

DESCRIPTION OF PLATES.

PLATE I. *Frontispiece.* Calla bed in which all the callas, 1,000 in number, were destroyed by the soft rot four years ago. Since that time three successful crops of the plant have been grown in this bed under the writer's direction, this being the third crop.

PLATE II. Fig. 1.—The organism that produces the soft rot of the calla, showing the form of the individual, the development in chains, and the presence of flagella (× 1,000). Fig. 2.—Development of colonies of the soft-rot organism on agar plates at 18° to 25° C. The organism with which these plates were inoculated had been kept dormant for two hundred and seventy-five days by withholding oxygen. Nearly all the colonies are round. Only a few show a slight tendency to radiate. Photographed three days after the plates were poured. (Natural size.) Figs. 3, 4, and 5.—These figures were made from agar plates which were inoculated with the same organism as figure 2, but after it had been for a longer time exposed to the air and had been transferred several times to fresh sterile beef broth. These plates were three days old and had been kept at a temperature of from 18° to 25° C.

PLATE III. Fig. 1.—Agar plate colony of the calla organism three days old at room temperature of about 20° C. The organism had been grown in beef broth previous to making the agar plate. (Natural size.) Fig. 2.—Agar plate colonies of the calla organism three days old. Grown at a temperature of 37° C. for three days, then kept for two days at about 20° C. (Natural size.) Fig. 3.—Tubes from which agar plates were poured photographed three days after pouring the plates; temperature, about 20° C. The agar was inoculated with a beef-broth culture of the calla organism. (Natural size.)

PLATE IV. Fig. 1, A.—Stab culture of the calla organism in neutral gelatin twenty-four hours after inoculation at 18° to 20° C. Fig. 1, B.—Stab culture of the calla organism in neutral gelatin three days old at 18° to 20° C. Fig. 1, C.—Stab culture of the calla organism in + 15 (acid) gelatin twenty-four hours after inoculation at 18° to 20° C. Fig. 2.—Raw eggplant in petri dish. Pieces 1 and 4 were inoculated with the calla organism, while pieces 2 and 3 were left for control. The photograph was made three days after inoculation.

PLATE V. Fig. 1.—Raw radish in petri dish. Nos. 2 and 3 were inoculated with the calla organism, while Nos. 1 and 4 were left for control. Photographed three days after inoculation. Fig. 2.—Side view of same plate nine days after inoculation. No. 2 was inoculated and No. 1 was left for control.

PLATE VI. A.—A cucumber inoculated with the calla organism. Photographed two days after inoculation, when the contents were soft throughout, except the spot near the stem end where the cucumber was inoculated. B.—A cucumber used for control; i. e., it was treated in the same manner as A, except that the calla organism was not applied to the punctures.

PLATE VII. Fig. 1.—Raw parsnip root in petri dish. The discolored pieces at right and left were inoculated, while the upper and lower pieces were left for control. Photographed three days after inoculation. (Natural size.) Fig. 2.—Raw carrot root three days after inoculation with the calla organism. Pieces 2 and 3 were inoculated, while pieces 1 and 4 were left for control. (Natural size.)

PLATE VIII. Fig. 1.—Raw turnip root in petri dish. The discolored pieces were inoculated with the calla organism, while the other pieces were left for control. As shown in this figure it is the center of the root that is most readily attacked by the organism. Fig. 2.—Green tomato fruit infected on the plant. The shriveled fruit shown at the base of the stem was inoculated with the calla organism. Photographed ten days after inoculation. The fruit at the left of the one inoculated remained sound in spite of the fact that it was in contact with the diseased fruit.

PLATE IX. Small calla plant, showing roots about 8 inches in length. The corm shows scars where it had evidently been attacked by the soft rot and had either recovered or the organism was dormant at the time the photograph was taken.

O

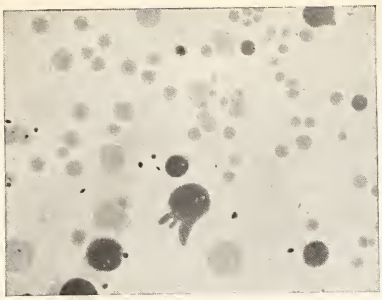

FIG. 2.—AGAR PLATE COLONIES.

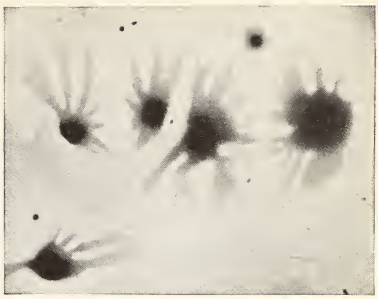

FIG. 3.—AGAR PLATE COLONIES.

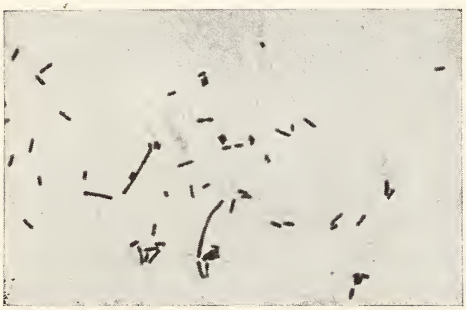

FIG. 1.—THE CALLA ROT ORGANISM × 1,000.

FIG. 4.—AGAR PLATE COLONIES.

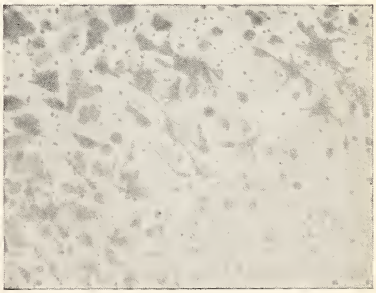

FIG. 5.—AGAR PLATE COLONIES.

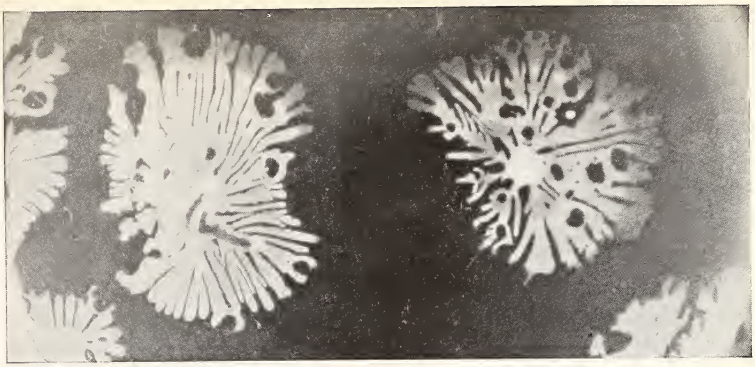

FIG. 1.—AGAR PLATE COLONIES OF THE CALLA ORGANISM GROWN AT 25° C.

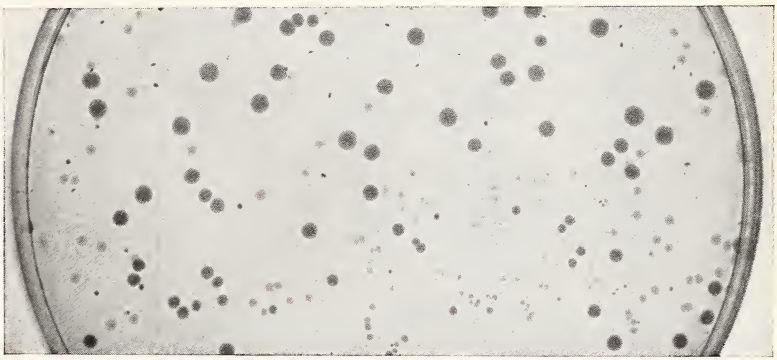

FIG. 2.—AGAR PLATE COLONIES OF THE CALLA ORGANISM GROWN AT 38° C.

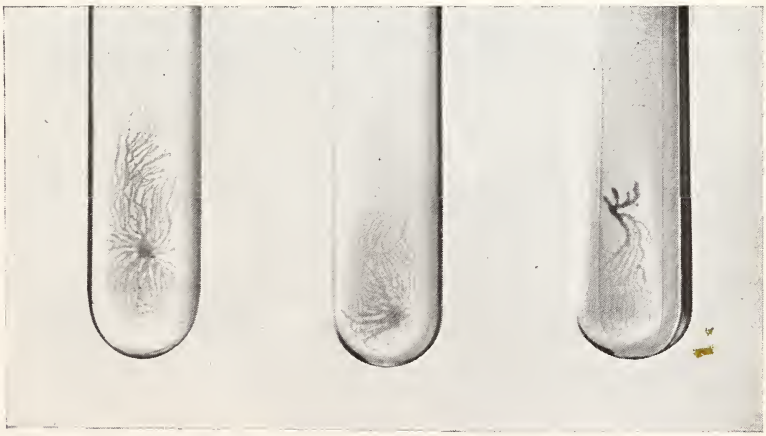

FIG. 3.—COLONIES OF THE CALLA ORGANISM IN TEST TUBES.

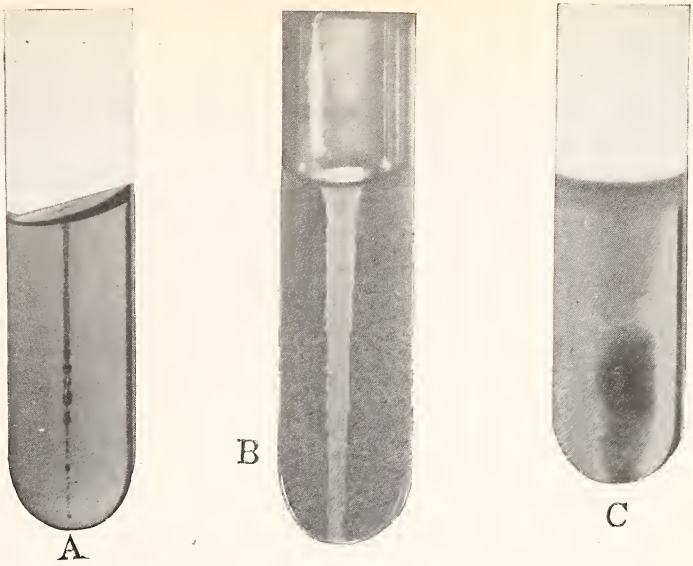

FIG. 1.—STAB CULTURES OF THE CALLA ORGANISM IN GELATIN.

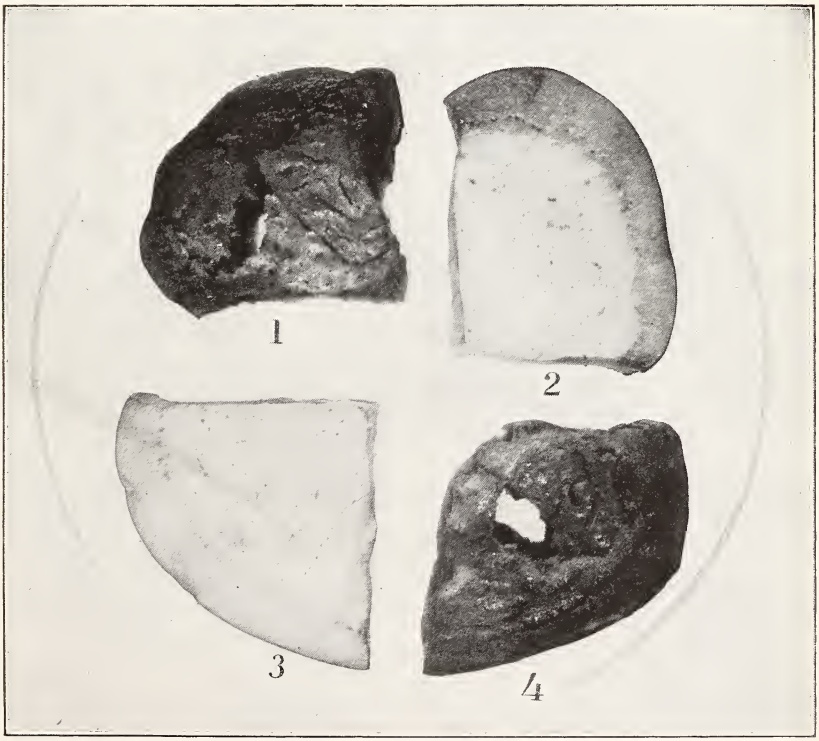

FIG. 2.—RAW EGGPLANT INOCULATED WITH THE CALLA ORGANISM. (NATURAL SIZE.)

FIG. 1.—RAW RADISH THREE DAYS AFTER INOCULATING PIECES 2 AND 3 WITH THE CALLA ORGANISM.

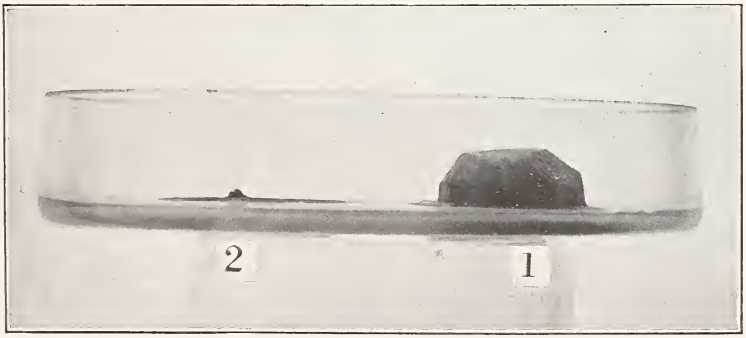

FIG. 2.—SIDE VIEW OF PIECES 1 AND 2 NINE DAYS AFTER INOCULATING NO. 2.

EFFECT OF CALLA ORGANISM ON CUCUMBER: A, INOCULATED; B, CONTROL.

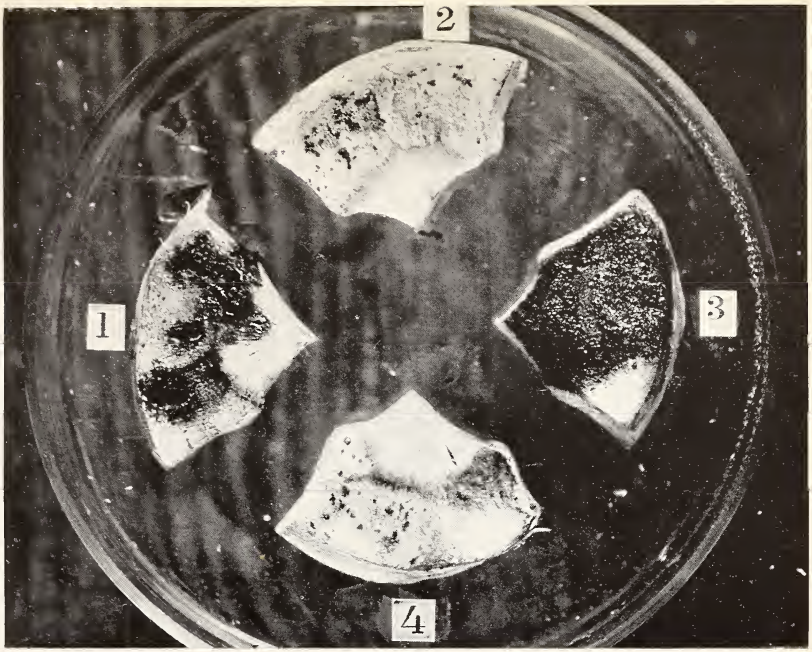

FIG. 1.—RAW PARSNIP THREE DAYS AFTER INOCULATING PIECES 1 AND 3.

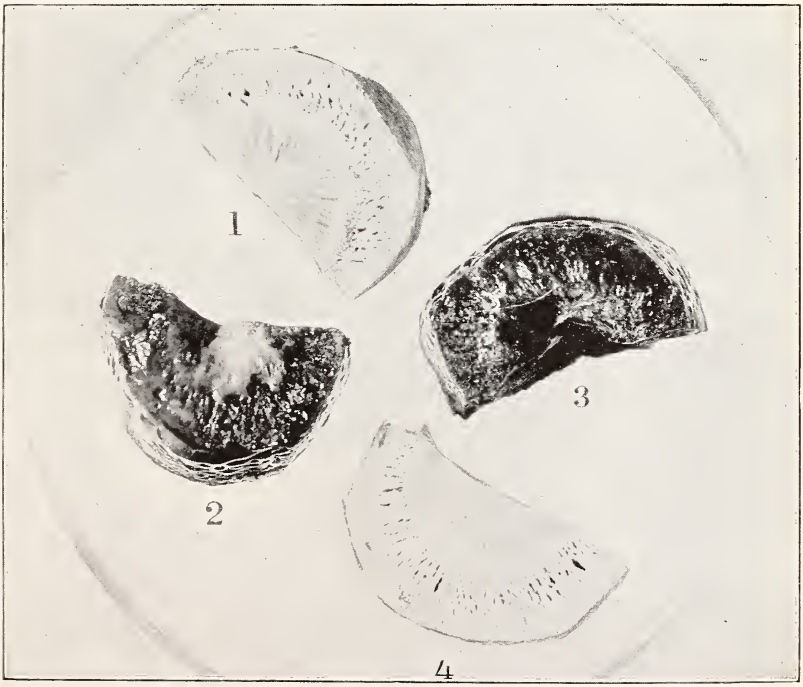

FIG. 2.—RAW CARROT THREE DAYS AFTER INOCULATING PIECES 2 AND 3.

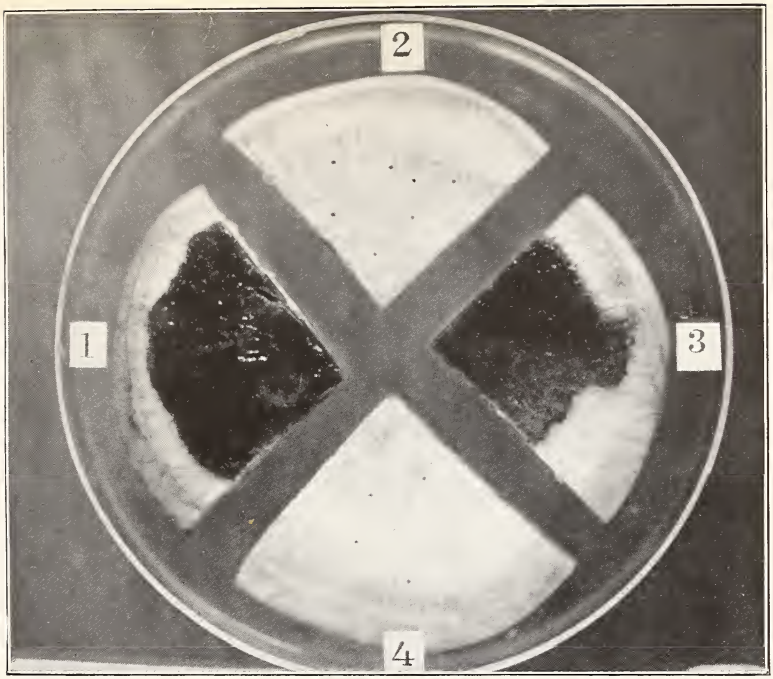

FIG. 1.—RAW TURNIP THREE DAYS AFTER INOCULATING PIECES 1 AND 3.

FIG. 2.—GREEN FRUIT AND BRANCH OF TOMATO: NO. 2, INOCULATED; NO. 1, CONTROL. (ONE-FOURTH NATURAL SIZE.)

SMALL CALLA PLANT, ABOUT TWO-THIRDS NATURAL SIZE.

[Continued from page 2 of cover.]

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